Optimal switching behavior, Just-in-time production

Jonas Maaskola
Institut für Mathematik und Informatik, Freie Universität Berlin
Zuse-Institut-Berlin

February 21, 2006

Contents

In the following two papers are reviewed. [Kalir et al., 2001] lends itself to an introductory discussion and will thus be presented first, while [Klipp et al., 2002] presents a more theoretical treatment of temporal optimality in metabolic pathways and will be discussed afterwards.

1 Introduction - Observing temporal optimality

[Kalir et al., 2001] presents an analysis of expression kinetics of the flagellar operons in *Escherichia coli*. The corresponding genes are organized in three operons as depicted in figure 1. There are two checkpoints in the expression of these operons. FlhDC, which is the only member of the first operon, enables expression of the second class of genes. Among the class 2 genes is FliA that turns on expression of the class 3 genes but this happens only after basal body-hook structures (BBH) are completed. This is implemented by FlgM, which binds and inhibits FliA. When BBH are completed, they export FlgM out of the cell, leaving FliA free to activate the class 3 operons.

1.1 Principle of Temporal Optimality

Underlying this review is a discussion of the principle of temporal optimality. Temporal optimality can be defined as maximizing a functional of a time-dependent function while respecting a given set of constraints. This will be defined more formally in discussing the second paper.

Temporal optimality of biological mechanisms is typically regarded as the result of a evolutionary process. Temporal optimality results from the limitedness of resources as well as the fact that parsimoniousness

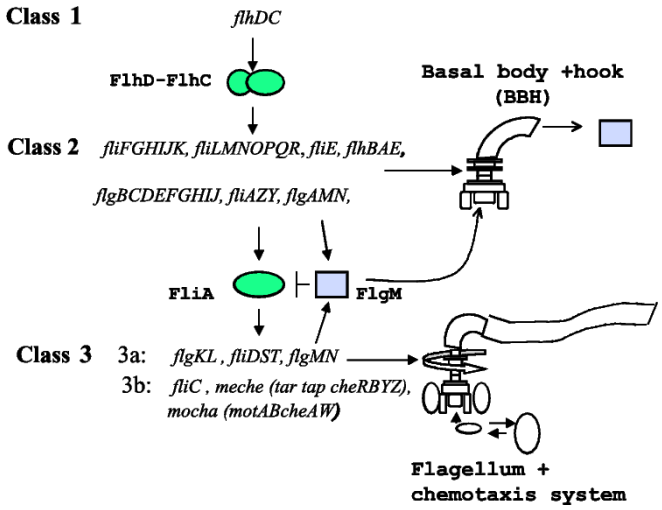

Figure 1: Hierarchy of flagellar operons in *Escheria coli*.

in spending resources can be improved upon by also making sure that enzymes or generally reactants are available right when they are needed so that a most effective relation between spending and gains is achieved. Temporal optimality can also be considered under the aspect of rivalry of individuals where those individuals that have the decisive mechanisms temporally optimized need not spend as much energy as their less favorably controlled intra-species contrahents for nutrition.

1.2 Inspecting temporal expression profiles

But as abstract principles can not directly be proven, but only motivated we turn again to the results of [Kalir et al., 2001] to see whether temporal optimality can really be observed.

The authors produced a number of reporter strains to follow the temporal expression profile of the genes involved in constructing the flagella in *E. coli*. As reporter technology fluoresence was chosen. The profiles are depicted in figure 2.

From the profiles we can see that the expression of the genes follows approximately the ordering into the three operons where flhD is the first one to rise in expression followed by the class 2 genes and only then by the class 3 genes. This can be even better observed in figure 3 where the expression profiles are normalized by their respective maximal fluorescence. In figure 3 it also becomes apparent how the checkpoint mechanism works. As there is residually expressed flhD in the pre-existing flagella condition the class 2 genes are expressed without delay. The same holds true for fliA and the class 3 genes. The authors also preformed a standard single-linkage clustering algorithm with a Euclidean metric on the expression profiles resulting in the dendrogram depicted to the left of figure 3. The ordering of the genes was determined by first considering each splitting from the top down and computing the average log fluoresence (normalized by the maximal fluoresence) for the two subtrees, $\log(f_1)$ and $\log(f_2)$. Next, by computing $t_i = -\int \log(f_i(t))\,dt$. The subtree with the smaller t_i was then positioned to the left.

1.3 Temporal optimality - can it be observed?

Having clearly observed this temporal ordering it is still necessary to argue why this would be an ideal ordering for the mechanism of flagellar gene expression and thus why this would be temporally optimal. That this is indeed the case can be observed in figure 4 where the functionality of the genes is shown. The early expressed genes consitute components of the flagellum that are more basal and located rather on the cytosolic side of the membrane with the later expressed genes coding for components that both located

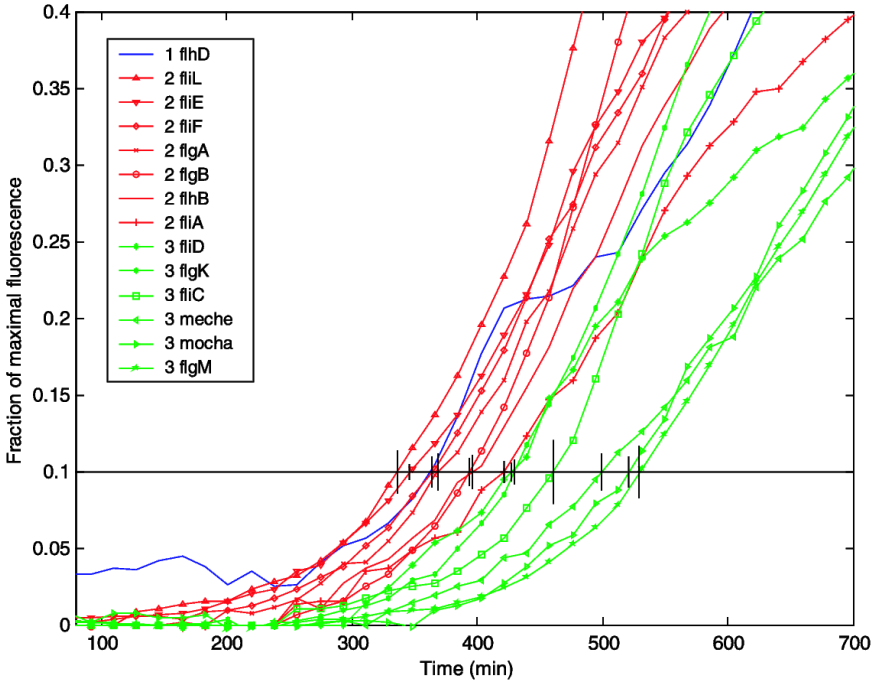

Figure 2: Fluorescence of flagella reporter strains as a function of time, normalized by the maximal fluorescence of each strain.

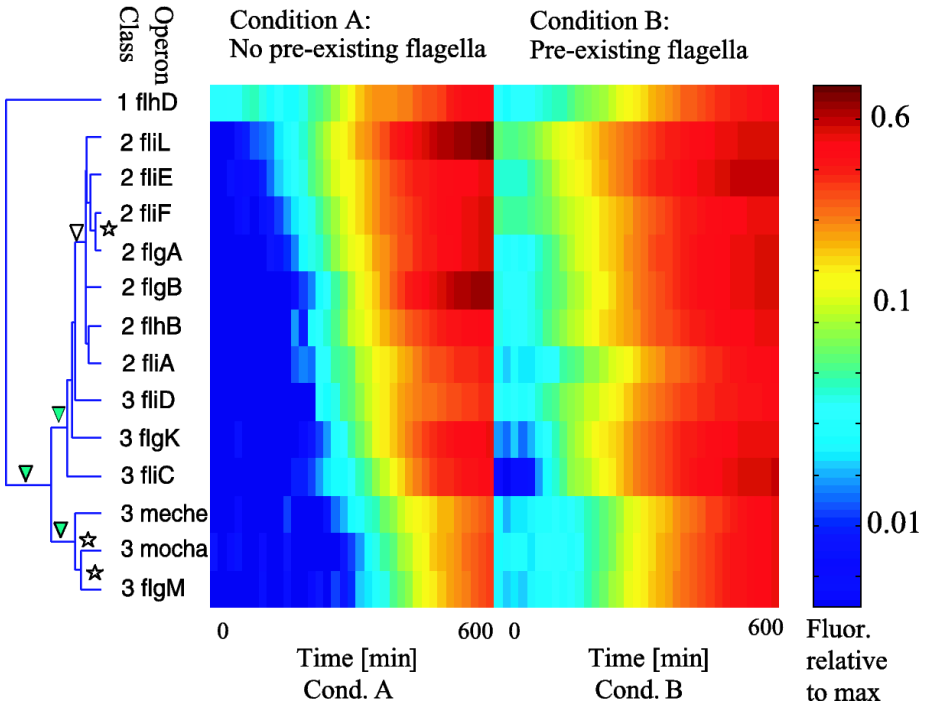

Figure 3: Fluorescence of flagella reporter strains as a function of time for two conditions, normalized by the maximal fluorescence of each strain scales from blue (low) to red (high).

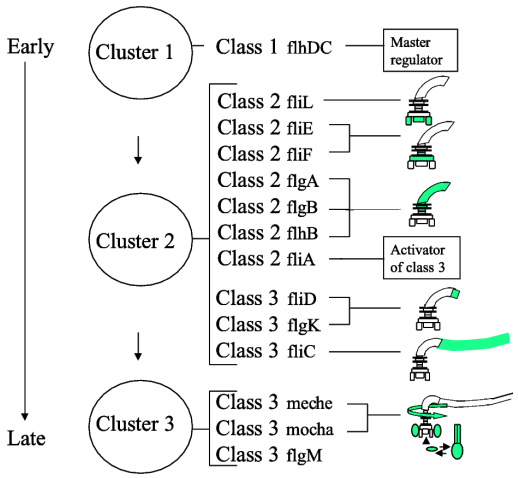

Figure 4: Resulting kinetic classification of the flagellar operons due to [Kalir et al., 2001].

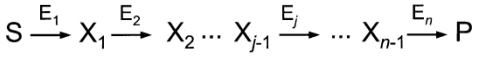

Figure 5: Simple unbranched reaction chain with linear kinetics of n reaction steps converting substrate S into product P.

further outside of the membrane and are later intergrated into the multi-protein complex of the flagellum. It seems obvious that expressing for example meche or mocha while they could not yet be assembled onto a preexisting complex consisting of class 2 gene products does not make so much sense as they can not unfold their utility as long as the other proteins are not produced yet. From this it seems reasonanble to argue that what we see here is an instance of temporal optimality in transcriptional organization.

2 Linear kinetics - Theoretical foundation and application

After having investigated the justification of a rather semantic definition of temporal optimality we take a look at [Klipp et al., 2002] where a more formal definition of the temporal optimality notion is given. The paper does not explicitely target the theory of temporal optimality but rather explains it via two examples of which the first one is an artificially designed system, whereas the second one is an application to a metabolic pathway. Both examples employ linear kinetics.

2.1 Theoretical model - Unbranched reaction chain

As an artificial system the model depicted in figure 5 is considered. It is a chain of n reaction steps each governed by linear kinetics. The system can be described through the following system equations:

$$\frac{dS}{dt} = -k_1 \cdot E_1 \cdot S \tag{1}$$

$$\frac{dX_i}{dt} = k_i \cdot E_i \cdot X_{i-1} - k_{i+1} \cdot E_{i+1} \cdot X_i \tag{2}$$

$$\frac{dP}{dt} = k_n \cdot E_n \cdot X_{i-1} \tag{3}$$

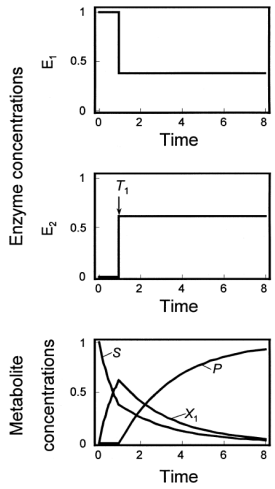

Figure 6: Optimal enzyme profiles and metabolite time courses for the linear metabolic pathway with one intermediary metabolite ($n = 2$).

The limitedness of resources is modelled here by the following constraint.

$$\sum_{i=1}^{n} E_i(t) = E_{\text{tot}} \tag{4}$$

The benefit that a well controlled individual experiences is modeled in the temporal optimality framework by the following performance functional.

$$\tau = \frac{1}{C} \int_0^\infty (C - P(t))\, dt \tag{5}$$

$$C = S|_{t=0} \tag{6}$$

$$\tau \rightarrow \text{MINIMUM} \tag{7}$$

Here C is the intial concentration of S and τ is subject to minimization. As τ is the integral over the not yet fully to P converted amount of S this preformance functional is depended on the time required such that the entire intial concentration of S has been converted to P. Thus τ seems to be a reasonable measure of performance. It can be expetced as resulting in a control over the mechanism that converts S as fast as possible to P.

As τ is a functional of the time-dependent function $P(t)$, which is subject to control via the above given system equations, it is necessary to decide on which Banach space to optimize the enzyme concentration profiles. Motivated from a simple example of a car that has to move from a place a to a place b subject to bounds on the maximal and minimal acceleration under the additional constraint that the intial and end velocity both have to be 0 it seems at first reasonable to choose the space of piecewise constant functions for the enzyme concentrations. Further decisions have to be made on the number of switching events. It also remains to be considered how such an ideal piecewise-constant control could really be realized in nature or to which approximation it is achieved, but this is of lesser concern to this paper.

2.1.1 One intermediary metabolite

At first we consider the case of only one intermediary metabolite. Thus $n = 2$. The solution to the optimization problem stated above was determined explicitely by the authors and is depicted in figure 6 We observe that the optimal control is realized by a one-switch process where in the first phase the entire enzyme concentration is attributed to E_1 and only in the second phase there is some concentration of E_2. Thus production of P only happens after the switch.

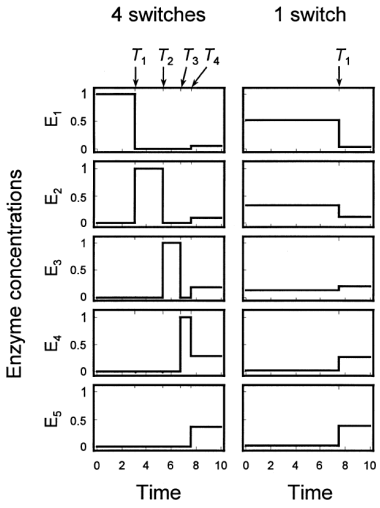

Figure 7: Optimal enzyme profiles and metabolite time courses for the linear metabolic pathway ($n = 5$).

2.1.2 Four intermediary metabolites

For the case of four intermediary metabolites ($n = 5$) the authors performed a numerical optimization of the vectors T_i and $E_i(j)$ describing the switching times and enzyme concentrations in the different phases respectively. The numerical optimization was performed by first explicitely solutving of system equations for $P(t)$ depending on T_i and $E_i(j)$, then explicitely calculating the transition time τ and finally minimizing τ by a steepest descent method.

The numerical solution to the optimazation problem for the linear metabolic pathway with four intermediary metabolites is depicted in figure 7. On the right hand side the optimal solution for the one switch case is given while the left hand side shows the optimal solution for the four switch case. The one switch case allots in the first phase ever decreasing amounts to the later enzymes while in the second phase the reverse holds true. In the four switch case we can observe a wave like profile moving through the enzymes. In the first phase the first enzyme is allotted the biggest amount, while the wave moves on in the second phase to the second enzyme and so on. There is some residual concentration allotted to all enzymes in the last phase while the last enzyme is assigned most.

Figure 8 shows in the upper part the minimal transition times for four intermediary metabolites as a function of the allowed switches. We observe that the minimal transition time decreases with the number of switches but only until the number of switches reaches that of the intermediary metabolites. In the lower half of the figure we see the minimal transition times for 0 to 9 intermediary metabolites as a function of the pathway length. The τ_{ref} curve depicts the optimal solution in the case of 0 switches, while the $\tau_{\min}$ labeled curve uses respectively as many switches as there are metabolites. Here we observe that with increasing pathway length the payoff of time-dependent optimality increases.

2.2 Application - Central metabolism of yeast

After having formulated a mathematical framework for the temporal optimality principle for the linear kinetics of metabolic control we next want to see whether such modeling can successfully be applied to real world biological systems. In [Klipp et al., 2002] the central metabolism of yeast is examined in search of temporal optimality properties. It is depicted in figure 9. As temporal optimality needs some reference time it was considered how the yeast central metabolism reacts to a diauxic shift.

For the system equations the interested reader is refered to the original paper because it would be tedious to reproduce them here and it seems also unnecessary. More interesting are the how the limitedness of

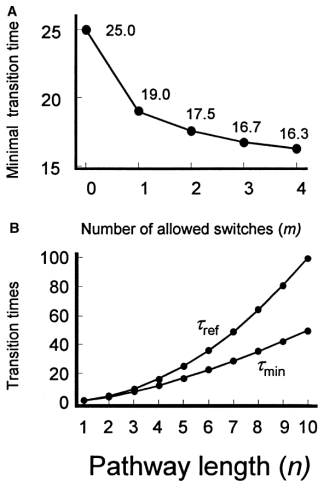

Figure 8: (A) Minimal transition times for 4 intermediary metabolites as a function of the allowed switches. (B) Minimal transition times for 0 to 9 intermediary metabolites as a function of the pathway length.

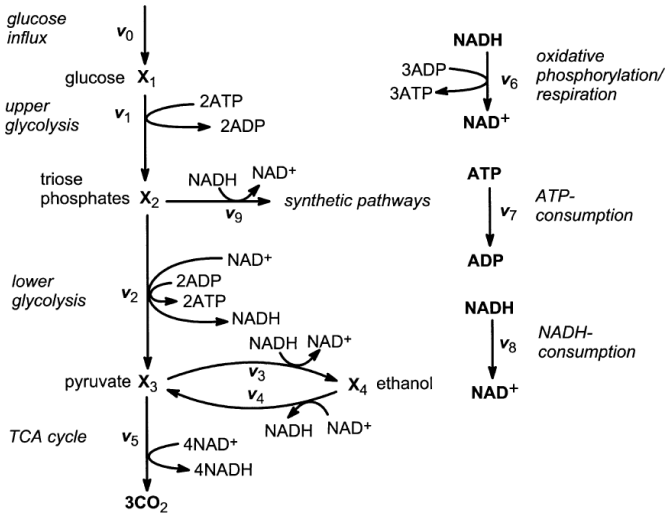

Figure 9: Skeleton model of the central metabolism of yeast. Groups of enzymes constituting pathways or functional parts of pathways are represented as single overall reactions.

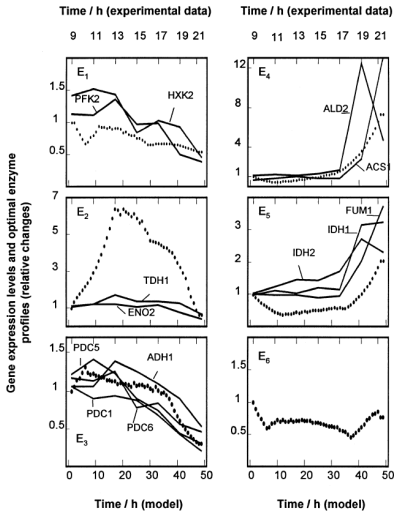

Figure 10: Optimal enzyme profiles ensuring a maximal survival time of yeast cells under conditions of a diauxic shift. Optimal enzyme profiles: dotted curves; Related gene expression profiles: solid curves

natural resources is modeled in this case. The constraints are:

$$NADH + NAD^{+} = const. \tag{8}$$

$$ATP + ADP = const. \tag{9}$$

$$\sum_{i=1}^{6} E_i(t) \leq E_{tot} \tag{10}$$

This models that the total amount of the metabolite $NADH$ and NAD, as well as that of ATP and ADP should not change. This is equal to requiring that they are only converted into each other. Also the total amount of enzyme is bounded above. The performance measure is modeled here according to the notion of survival time.

$$\vartheta = t\,\Theta\left(ATP - ATP_c\right)\,\Theta\left(NADH - NADH_c\right) \tag{11}$$

$$\Theta\left(x\right) = \begin{cases} 1 & \text{if} \quad x \geq 0 \\ 0 & \text{if} \quad x < 0 \end{cases} \tag{12}$$

$$\vartheta \rightarrow \text{MAXIMUM} \tag{13}$$

Survival time is expressed here by enforcing that the concentrations of the two energy equivalents of the cell are as long as possible over a defined threshold.

2.2.1 Temporally optimal solutions

Optimal enzyme profiles are calculated in this case using a genetic algorithm. Figure 10 shows these solutions as dotted curves while concentration profiles of genes that are related according to literature are shown as solid curves. The qualitative similarity is surprising considering that only linear kinetics is employed. Altogether reproducing these curves in an almost quantitative way is a nice affirmation of the supposed temporal optimality property of this system.

The resulting time-courses of the metabolites ATP, $NADH$ and ethanol are depicted in figure 11. In this figure time-independent, single switch and globally optimal solutions for the model are shown. From this the maximal survival times are clearly visible. How the optimally controlled solution copes to stay alive longer seems to follow from its buffering carbon energy equivalent species in ethanol. The ATP curve looks

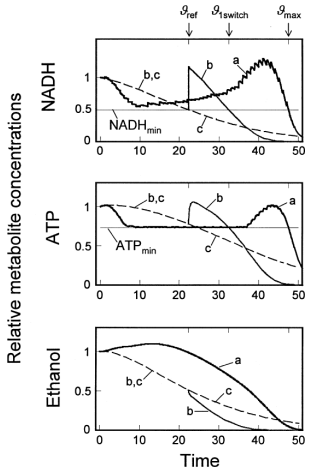

Figure 11: Calculated time-courses of some important metabolites oy yeast at enzyme profiles ensuring a maximal survival time of yeast. **a** optimal enzyme profiles; **b** one optimal single switch of enzyme acitvities; **c** time-independent enzyme concentrations

a little suspicious; it might be that is an artifact resulting from the hard gate function Θ employed in the maximization procedure. If survival is really to be modeled with such a hard gate function then the resulting curve for ATP should not be considered robust to little perturbations.

3 Conclusions

The two papers showed that temporal optimality can be observed in nature. [Kalir et al., 2001] demonstrated it on an argumentative level while [Klipp et al., 2002] modeled the temporal optimality in terms of a mathematical framework that was first applied to a theoretical model and subsequently to a real world system. The argumentation in [Kalir et al., 2001] sounds sensible, yet linguisitic argumentation is often not formally convincing and thus the [Klipp et al., 2002] paper is an adequate continuation. It achieved a quite convincing prediction of several enzyme profiles regulating important reactions of the central yeast metabolism facing a diauxic shift. On these grounds it can thus be argued that we really have observed temporal optimality in this system. We may even be tempted to extrapolate to formulate that the temporal optimality principle is recurring in many instances in biology. This would of course only hold as long as no contradictions are shown and no better explanations are available.

References

[Kalir et al., 2001] Kalir, S., McClure, J., Pabbaraju, K., Southward, C., Ronen, M., Leibler, S., Surette, M. G., and Alon, U. (2001). Ordering genes in a flagella pathway by analysis of expression kinetics from living bacteria. *Science*, 292(5524):2080–3.

[Klipp et al., 2002] Klipp, E., Heinrich, R., and Holzhütter, H.-G. (2002). Prediction of temporal gene expression: Metabolic optimization by re-distribution of enzyme activities. *Eur J Biochem*, 269(22):5406–5413.